X-linked severe combined immunodeficiency (XSCID) is caused by mutations of the common gamma chain (γc) and usually characterized by the absence of T and natural killer (NK) cells. Here, we report an atypical case of XSCID presenting with autologous T and NK cells and Omenn syndrome-like manifestations. The patient carried a splice-site mutation (IVS1+5G>A) that caused most of the mRNA to be incorrectly spliced but produced normally spliced transcript in lesser amount, leading to residual γc expression and development of T and NK cells. The skin biopsy specimen showed massive infiltration of revertant T cells. Those T cells were found to have a second-site mutation and result in complete restoration of correct splicing. These findings suggest that the clinical spectrum of XSCID is quite broad and includes atypical cases mimicking Omenn syndrome, and highlight the importance of revertant mosaicism as a possible cause for variable phenotypic expression. (Blood. 2008;112: 1872-1875)

Methods

Patient

A 5-month-old Japanese boy from nonconsanguineous parents was hospitalized because of failure to thrive, protracted diarrhea, and hypoproteinemia. He developed generalized erythematous rash and alopecia at 1 month of age (Figure 1A,B), followed by persistent cough, fever, hepatosplenomegaly, eosinophilia, hypogammaglobulinemia, elevated serum IgE, and activated/oligoclonal T cells.1,2 The genes responsible for OS include RAG1, RAG2, Artemis, RMRP, and IL7RA.3-8 Clinical manifestations resembling OS were demonstrated in cases of severe combined immunodeficiency (SCID) with maternal T-cell engraftment and of atypical DiGeorge syndrome.9,10 In addition, we have recently reported an X-linked SCID (XSCID) patient with massive skin infiltration of natural killer (NK) cells, resulting in OS-like manifestations.11 Here, we describe another case of XSCID mimicking OS. The patient carried a splice site mutation that allowed development of peripheral T and NK cells, and showed revertant T-cell mosaicism caused by a second-site mutation predominately in the skin. Both of these findings may have contributed to his phenotypic variation of XSCID.

Results and discussion

The patient’s clinical findings were reminiscent of OS. However, he showed neither mutation in the RAG genes (data not shown) nor severely restricted TCR repertoire (Figure 1E,F). Microsatellite analysis ruled out maternal T-cell engraftment (Figure 1G). Because of possible X-linked inheritance in this family and our previous experience,11 we assessed γc expression. FACs analysis clearly demonstrated defective γc expression on his lymphocytes and lymphocyte subpopulations, leading to the diagnosis of XSCID (Figure 1H). The patient had a G to A substitution at the fifth nucleotide of intron 1 of the IL2RG (IVS1+5G>A) that was previously listed in the IL2RG database (http://research.nhgri.nih.gov).
nih.gov/apps/scid/IL2RGbase.shtml), and that caused most of the mRNA to be incorrectly spliced but produced, in lesser amount, normally spliced wild-type transcript (Figure 2A-D). When we analyzed such sequences in subcloned RT-PCR products obtained from his peripheral blood mononuclear cells, the wild-type sequence was detected in 15% of 20 clones. Accordingly, his
lymphocytes showed the residual γc protein that likely permitted T- and NK-cell development to occur. Results were obtained from the study in another case of atypical XSCID with poorly functioning peripheral T cells.16

The presence of circulating T cells offered us a unique opportunity to establish T-cell lines from XSCID. Unexpectedly, FACS analysis showed a significant fraction of cells with normal γc expression among his T-cell lines (Figure 2E). Therefore, sequence analysis was performed using the cloned T-cell lines. We found the IVS1+5G>A in DNA from the γc-defective clone (#3-4), whereas DNA from the γc-expressing clone (#3-1) showed coexistence of the IVS1+5G>A and a second-site mutation (IVS1+29G>A), which was located at position +1 of the cryptic 5’ splice site (Figure 2F). Analysis of 100 normal chromosomes demonstrated the absence of the IVS1+5G>A and the IVS1+29G>A. The IVS1+29G>A was found to suppress the effect of the IVS1+5G>A and result in complete restoration of correct splicing that was consistent with the restored γc expression on the cell surface (Figure 2G). To determine whether the IVS1+29G>A was present in vivo, direct sequencing was performed with his various DNA samples. A peak of the IVS1+29G>A was only detectable in his skin specimen, but was absent among circulating CD4+ and CD8+ T and CD19+ B cells (Figure 2H). When we analyzed the sequence in subcloned PCR products obtained from the skin, the IVS1+29G>A was detected in 12.9% of 31 clones. RT-PCR and GeneScan analysis demonstrated that a peak of the normal-sized transcript but not the mutated one was evident in the skin. Because IL2RG mRNA is expressed only in hematopoietic cells, these findings suggest that the majority of skin-infiltrating lymphocytes carry the IVS1+29G>A, thus indicating revertant cells (Figure 2I).

Although revertant γc expression would confer selective advantage in vivo to T- and NK-cell progenitors,17 we could not detect the γc-expressing T cells in his circulation. This lack of in vivo selective advantage is in contrast to a previously described XSCID patient with revertant T-cell mosaicism in whom a reasonably diversified TCR repertoire was generated from a single T-cell progenitor, resulting in a mild clinical course.18,19 The reasons underlying these findings are presently unclear. It is possible that the substantial numbers of activated peripheral T cells with defective γc expression that were not seen in the previous case19 might have competed with the revertants, reduced the abilities of selective advantage of γc expression, and provided insufficient time for the reversion to reach the detection threshold in our patient. On the other hand, selective proliferation of revertant CD8+ T cells with TCR VB2 in the skin (Figure 2J,K) may be due to a clonal expansion in response to infections or autoantigens and be involved in pathogenesis of his skin lesions. However, his complicated condition including the presence of autologous γc-defective T and NK cells in the peripheral blood and the expansion of revertant γc-expressing T cells in the skin does not allow us to draw clear conclusion about which aspects are responsible for the development of OS phenotype. Because recent studies in OS patients and murine models have indicated that impaired immune tolerance and
defective immune regulation play important roles in the pathophysiology of OS, the highly elevated numbers of activated, γc-defective T cells with a moderate restricted TCR repertoire may have more contributed to the OS phenotype in our patient. If this is the case, it is possible that any SCID diseases having a profound but incomplete block in T-cell development could result in OS.

In summary, our studies indicate that the clinical spectrum of XSCID is broad and includes immunodeficient patients mimicking OS. In addition, the detection of the revertant T cells caused by the second-site suppressor mutation makes our case a second example of genetic reversion in XSCID and highlights the importance of somatic revertant mosaicism as a possible cause for atypical clinical presentations.

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References


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Authorship

Contribution: T.W. and A.Y. participated in designing and performing the research; T.T., Y.N., M.N., M.O., Y.K., and S.K. analyzed data; M.Y., M.I., and K.K. made clinical contributions and provided critical paper review; T.W. wrote the paper; and all authors checked the final version of the manuscript.

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Detection of T lymphocytes with a second-site mutation in skin lesions of atypical X-linked severe combined immunodeficiency mimicking Omenn syndrome

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